Introduction

Apolipoprotein E (apo E) is an important structural constituent of serum chylomicrons, very low-density lipoproteins, and high-density lipoproteins (HDL) and plays a critical role in lipoprotein metablism, where it can facilitate the clearance of remnant lipoprotein and cellular efflux of cholesterol[1]. Apo E has three polymorphisms, $\varepsilon 2$, $\varepsilon 3$, and $\varepsilon 4$, which affect lipoprotein metabolism and atherosclerosis[2]. The $\varepsilon 4$ allele is associated with higher low-density lipoprotein (LDL) cholesterol levels than the other alleles and with a higher incidence of coronary heart disease[3]. Apo E4 is also shown to be involved in the development of Alzheimer's disease[4], while homozygosity for apo E2 is associated with the development of type III hyperlipidemia[5].

We also studied the *MTHFR* gene because its polymorphisms affect serum homocysteine levels and homocysteine is also associated with cardiovascular disease and Alzheimer's disease[6-9]. An elevated homocysteine level is associated with coronary heart disease and the C677T polymorphism in the *MTHFR* gene results in reduced *MTHFR* enzyme activity and reduced methylation of homocysteine to methioneine resulting in mild hyperhomocysteinemia[10]. Although several studies have examined the incidence of *APOE* and *MTHFR* polymorphisms[8, 11], these has been no large-scale study to determine the incidence of *APOE* and *MTHFR* polymorphisms and their association with lipoprotein profiles and homocysteine levels in the general Japanese population. In 2000, we conducted a lipid survey in the Japanese population, 12,839 people all over the country. In this survey, we examined *APOE* and *MTHFR* gene polymorphisms to determine the incidence of each and its relationship with lipid profiles and homocysteine levels in the Japanese.

Methods

Design and Data Collection

This work is part of Serum Lipid Level Survey 2000 from various parts of Japan. The Ethics committee, Graduate School and Faculty of Medicine, Kyoto University approved the study protocol and all subjects provided written informed consent for participation in the gene analysis. The handling of DNA samples followed the guidelines from the Ministry of Health, Labor, and Welfare. In Serum Lipid Survey 2000, a total of 12,839 subjects were recruited at 36 hospitals across the country. The subjects in the present study were participants in the survey at 9 hospitals from whom informed content for genotyping was sought. Of the 12,839 subjects, 2,267 (17.7%) with no lipid-altering medication were randomly selected for the present study. Among the 2,267 participants, we examined serum homocysteine levels and *MTHFR* gene polymorphisms in 505 participants.

Laboratory Methods

All serum and blood samples were obtained in the fasting state. All lipid and other analyses were conducted on venous blood samples within one week of collection at BML (Saitama, Japan). Serum cholesterol and TG levels were measured by enzymatic assay. HDL-cholesterol and LDL-cholesterol levels were measured enzymatically with a kit from Daiichi Kagaku Co. Ltd. (Tokyo, Japan). The results of lipid analyses in the four surveys were indirectly standardized according to the criteria of the CDC Lipid Standardization Program[12]. The serum homocysteine level was assayed by high performance liquid chromatography with fluorescent detection as described by Ubbink *et al.*[13]. DNA was extracted with a QIAamp DNA blood kit (Qiagen, Hilden, Germany).

Detection of gene Mutations by Invader® Assay

We used the Invader® assay to screen for mutations of the *APOE* and *MTHFR* genes, as previously described. In brief, the probe/Invader®/MgCl2 mixture was prepared by combining 3 μL of primary probe/Invader® mix and 5 μL of 22.5 mM MgCl2 per reaction. The primary probes/Invader® mixture contained 3.5 μmol/L wild primary probe, 3.5 μmol/L mutant primary probe, 0.35 μmol/L Invader® oligonucleotide, and 10 mmol/L MOPS. Eight microliters of primary probe/Invader®/MgCl2 mixture was added per well of a 96-well plate. Seven microliters of 5 fmol/L synthetic target oligonucleotides, 10 μg/mL yeast tRNA (no target blank), and genomic DNA (15 ng/μL) were added, and denatured by incubation at 95℃ for 10 min. After 15 μL of mineral oil (Sigma, St. Louis, MO) was overlayed into all reaction wells, the plate was incubated isothermally at 63℃ for 4 h in a DNA thermalcycler (PTC-200; MJ Research, Watertown, MA) and then kept at 4℃ until fluorescence were measured. The intensity of the fluorescence was measured with a fluorescence microtiter plate reader (Cytoflour 4000; Applied Biosystems) with excitation at 485 nm/20 nm (Wavelength/Bandwidth) and emission at 530 nm/25 nm for FAM; and excitation at 560 nm/20 nm and emission at 620 nm/40 nm for RED. The genotyping was analyzed by calculating the ratio of net counts with wild primary probe to net counts with mutant primary probe. The probes used in this study were designed and synthesized by Third Wave Technologies, Inc (Madison, WI).

Data Analyses

Differences in means were evaluated with an analysis of variance. The analysis was performed with the statistical Package for Social Sciences (SPSS Japan Inc. ver. 11.5, Tokyo, Japan).

Results

We investigated the frequency and phenotypic association of *APOE* gene polymorphisms of 2,267 subjects. We found that the SNPs were in Hardy-Weinberg equilibrium. As previously described, the mean age, total cholesterol, TG, HDL-cholesterol, and LDL-cholesterol levels in this population were similar to the levels for all 12,839 patients in Serum Lipid Survey 2000[14]. We also found that the medians of total, LDL-, and HDL-cholesterol levels did not differ appreciably from the means, thereby excluding gross right-hand tailing of the distribution (data not shown). These data indicate that the participants in the gene analysis are representative of the general Japanese population.

The genotype and allelic frequency of *APOE* polymorphisms are presented in **Table 1**. The frequency of the $\varepsilon 2$, $\varepsilon 3$, and $\varepsilon 4$ alleles was 4.2, 85.3, and 10.5%, respectively. As in other studies, the genotypes $\varepsilon 2\varepsilon 2$, $\varepsilon 2\varepsilon 4$, and $\varepsilon 4\varepsilon 4$ were quite rare. High frequencies of the $\varepsilon 3$ allele are also found in Chinese, but the frequency is lower in Caucasians[15].

Table 1. Genotype and allele frequency of *APOE* gene in Japanese.

genotype	*n*	%	alleles	*n*	%
$\varepsilon 2/\varepsilon 2$	9	0.4	$\varepsilon 2$	192	4.2
$\varepsilon 2/\varepsilon 3$	155	6.8	$\varepsilon 3$	3,868	85.3
$\varepsilon 2/\varepsilon 4$	19	0.8	$\varepsilon 4$	474	10.5
$\varepsilon 3/\varepsilon 3$	1,653	72.9			
$\varepsilon 3/\varepsilon 4$	407	18.0			
$\varepsilon 4/\varepsilon 4$	24	1.1			

We next examined the association of the *APOE* genotype and lipid profiles in these participants. As shown in **Table 2**, all the lipid parameters and blood glucose differed significantly among these genotypes by ANOVA. Total cholesterol, triglyceride, HDL-cholesterol, LDL-cholesterol, and RLP-cholesterol levels were different among the groups. The *p* values are shown in the right column. According to the post-hoc analysis, the total cholesterol level was significantly lower for genotype $\varepsilon 2\varepsilon 2$ than $\varepsilon 4\varepsilon 4$ and genotype $\varepsilon 2\varepsilon 3$ than $\varepsilon 3\varepsilon 3$, $\varepsilon 2\varepsilon 4$, or $\varepsilon 4\varepsilon 4$. The HDL-cholesterol level was significantly higher for $\varepsilon 2\varepsilon 3$ than $\varepsilon 2\varepsilon 4$. The LDL-cholesterol level was significantly lower for genotypes $\varepsilon 2\varepsilon 2$ and $\varepsilon 2\varepsilon 3$ than for $\varepsilon 3\varepsilon 3$, $\varepsilon 3\varepsilon 4$, and $\varepsilon 4\varepsilon 4$. The RLP-cholesterol level was significantly higher for $\varepsilon 2\varepsilon 2$ than $\varepsilon 2\varepsilon 3$, $\varepsilon 3\varepsilon 3$, $\varepsilon 3\varepsilon 4$, or $\varepsilon 4\varepsilon 4$ and for genotype $\varepsilon 2\varepsilon 4$ than $\varepsilon 2\varepsilon 3$, $\varepsilon 3\varepsilon 3$, or $\varepsilon 3\varepsilon 4$, although there was no significant difference in triglyceride levels according to the post-hoc analysis. Blood glucose or age did not differ significantly among the groups.

We next examined the association of the *MTHFR* C667T polymorphism with serum homocysteine levels in 505 samples randomly selected from 2,267 samples. As shown in **Table 3**, the incidence of the CC, CT, and TT genotypes was 33.9, 46.1, and 20.0%, respectively. The TT genotype was significantly associated with higher homocysteine levels in men and women, and statistical significance was found between CC and TT and between CT and TT by a post-hoc analysis. However, the difference was more prominent in men.

Discussion

There, we have shown in a large-scale study, the

Table 2. Mean of serum lipid levels and blood glucose in each genotype of *APOE* in Japanese.

	$\varepsilon 2/\varepsilon 2$	$\varepsilon 2/\varepsilon 3$	$\varepsilon 2/\varepsilon 4$	$\varepsilon 3/\varepsilon 3$	$\varepsilon 3/\varepsilon 4$	$\varepsilon 4/\varepsilon 4$	total	*p* value
	mean ± SEM	mean ± SEM	mean ± SEM	mean ± SEM	mean ± SEM	mean ± SEM	mean ± SEM	
T-cho	165.0 ± 23.8	189.7 ± 3.00	202.9 ± 12.6	201.8 ± 0.92	206.8 ± 1.95	223.3 ± 9.18	202.1 ± 0.81	<0.0001
TG	171.4 ± 52.8	118.8 ± 8.55	189.0 ± 53.3	117.0 ± 2.41	128.0 ± 5.16	127.9 ± 18.9	119.8 ± 2.13	0.023
HDL-c	51.2 ± 9.51	63.6 ± 1.92	53.0 ± 3.07	59.8 ± 0.41	58.0 ± 0.82	61.9 ± 3.48	59.7 ± 0.36	0.007
LDL-c	70.5 ± 5.63	101.9 ± 2.75	117.2 ± 8.07	118.5 ± 0.91	120.5 ± 1.93	131.5 ± 7.97	117.7 ± 0.79	<0.0001
RLP-c	22.9 ± 1.15	4.4 ± 0.37	12.5 ± 7.59	4.7 ± 0.17	5.2 ± 0.33	4.1 ± 0.58	4.8 ± 0.15	<0.0001
FBS	121.3 ± 19.5	104.7 ± 3.27	110.6 ± 9.37	103.9 ± 0.94	103.3 ± 2.17	88.6 ± 2.54	103.9 ± 0.83	0.461
age	52.8 ± 10.1	49.5 ± 2.11	50.8 ± 53.2	46.7 ± 0.69	47.4 ± 1.30	43.2 ± 4.61	47.1 ± 0.58	0.659

T-cho: total cholesterol (mg/dL), TG: triglyceride (mg/dL), HDL-c: HDL-cholesterol (mg/dL), LDL-c: LDL-cholesterol (mg/dL), RLP-c: remnant-like particles cholesterol (mg/dL), FBS: fasting blood sugar (mg/dL), SEM: standard error of the mean

Table 3. Genotype frequency of the *MTHFR* gene and its association with serum homocysteine levels in Japanese.

total					
genotype	*n*	%	mean	SEM	
CC	171	33.9	10.9	0.3	$p<0.001$
CT	233	46.1	11.6	0.24	
TT	101	20.0	15.7	1.23	
total	505	100	12.2	0.29	
male					
genotype	*n*	%	mean	SEM	
CC	92	33.6	10.7	0.36	$p<0.001$
CT	132	48.2	12.9	0.35	
TT	50	18.2	19.8	2.41	
total	274	100	13.4	0.52	
female					
genotype	*n*	%	mean	SEM	
CC	79	34.2	10.2	0.43	$p=0.005$
CT	101	43.7	10.1	0.27	
TT	51	22.1	11.9	0.57	
total	231	100	10.5	0.23	

SEM: standard error of the mean

frequency of the *APOE* genotype in the Japanese and its association with serum lipid levels. Frequencies of *APOE* genotypes are highly heterogenous among various populations. Epidemiological data indicate that the frequency of the $\varepsilon 3$ allele is higher in Japanese and Chinese than in Caucasians, while the frequency of the $\varepsilon 4$ allele is lower in Asians than Caucasians[5, 16]. Our data indicate that the frequency of the $\varepsilon 3$ allele is quite consistent with previous reports in Japanese[8, 11, 16, 17], and is slightly higher than that of Icelandic and Hungarian populations and much higher than that in the Finnish population[15].

Our study confirmed that the $\varepsilon 4$ allele is associated with higher, and the $\varepsilon 2$ allele is associated with lower, LDL cholesterol levels. Although there was a trend for individuals with the genotypes $\varepsilon 2/\varepsilon 4$ and $\varepsilon 2/\varepsilon 2$ to have higher triglyceride levels, it was not statistically significant by a post-hoc analysis, probably because triglyceride levels are highly variable. However, individuals with $\varepsilon 2/\varepsilon 4$ and $\varepsilon 2/\varepsilon 2$ had significantly higher RLP-cholesterol levels than did those with the other genotypes, indicating that RLP-cholesterol might be better correlated with *APOE* genotype. Although in this study we could not compare the body mass index of $\varepsilon 2/\varepsilon 2$ homozygotes, it would be intriguing to know whether individuals with the $\varepsilon 2/\varepsilon 4$ and $\varepsilon 2/\varepsilon 2$ genotypes have metabolic abnormalities, such as abdominal obesity and insulin resistance, because they have higher triglyceride, RLP-cholesterol, and blood glucose levels.

Elevated levels of homocysteine have been considered a risk for cardiovascular disease. Our study is consistent with other studies that show higher homocysteine levels in people with the TT genotype. However, the relationship between the C677T *MTHFR* polymorphism and cardiovascular disease is still controversial. Because our study population is made up of healthy volunteers, a prospective study is necessary to determine which genotype is associated with cardiovascular risk.

In summary, we have provided the largest database of gene polymorphisms related to lipid metabolism and homocysteine in the general Japanese population. A prospective study is necessary to determine the contribution of these gene polymorphisms to cardiovascular risk in Japanese.

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References

1) Curtiss LK and Boisvert WA: Apolipoprotein E and atherosclerosis. Curr Opin Lipidol, 2000; 11:243-251
2) Davignon J, Gregg RE, and Sing CF: Apolipoprotein E polymorphism and atherosclerosis. Arteriosclerosis, 1988; 8:1-21
3) Wang XL, McCredie RM, and Wilcken DE: Polymorphisms of the apolipoprotein E gene and severity of coronary artery disease defined by angiography. Arterioscler Thromb Vasc Biol, 1995; 15:1030-1034
4) Katzman R: Apolipoprotein E and Alzheimer's disease. Curr Opin Neurobiol, 1994; 4:703-707
5) Rall SC Jr. and Mahley RW: The role of apolipoprotein E genetic variants in lipoprotein disorders. J Intern Med, 1992; 231:653-659
6) Boushey CJ, Beresford SA, Omenn GS, and Motulsky AG: A quantitative assessment of plasma homocysteine as a risk factor for vascular disease. Probable benefits of increasing folic acid intakes. JAMA, 1995; 274:1049-1057
7) Graham IM, Daly LE, Refsum HM, Robinson K, Brattstrom LE, Ueland PM, Palma-Reis RJ, Boers GH, Sheah-

an RG, Israelsson B, Uiterwaal CS, Meleady R, McMaster D, Verhoef P, Witteman J, Rubba P, Bellet H, Wautrecht JC, de Valk HW, Sales Luis AC, Parrot-Rouland FM, Tan KS, Higgins I, Garcon D, and Andria G: Plasma homocysteine as a risk factor for vascular disease. The European Concerted Action Project. Jama, 1997; 277:1775-1781

8) Ou T, Yamakawa-Kobayashi K, Arinami T, Amemiya H, Fujiwara H, Kawata K, Saito M, Kikuchi S, Noguchi Y, Sugishita Y, and Hamaguchi H: Methylenetetrahydrofolate reductase and apolipoprotein E polymorphisms are independent risk factors for coronary heart disease in Japanese: a case-control study. Atherosclerosis, 1998; 137:23-28
9) Clarke R, Smith AD, Jobst KA, Refsum H, Sutton L, and Ueland PM: Folate, vitamin B12, and serum total homocysteine levels in confirmed Alzheimer disease. Arch Neurol, 1998; 55:1449-1455
10) Brattstrom L, Wilcken DE, Ohrvik J, and Brudin L: Common methylenetetrahydrofolate reductase gene mutation leads to hyperhomocysteinemia but not to vascular disease: the result of a meta-analysis. Circulation, 1998; 98:2520-2526
11) Shinomiya S, Sasaki J, Kiyohara C, Tsuji E, Inoue H, Marugame T, Handa K, Hayabuchi H, Hamada H, Eguchi H, Fukushima Y, and Kono S: Apolipoprotein E genotype, serum lipids, and colorectal adenomas in Japanese men. Cancer Lett, 2001; 164:33-40
12) Johnson CL, Rifkind BM, Sempos CT, Carroll MD, Bachorik PS, Briefel RR, Gordon DJ, Burt VL, Brown CD, Lippel K, and Cleeman JI: Declining serum total cholesterol levels among US adults. The National Health and Nutrition Examination Surveys. Jama, 1993; 269:3002-3008
13) Ubbink JB, Hayward Vermaak WJ, and Bissbort S: Rapid high-performance liquid chromatographic assay for total homocysteine levels in human serum. J Chromatogr, 1991; 565:441-446
14) Arai H, Yamamoto A, Matsuzawa Y, Saito Y, Yamada N, Oikawa S, Mabuchi H, Teramoto T, Sasaki J, Nakaya N, Itakura H, Ishikawa Y, Ouchi Y, Horibe H, Egashira T, Hattori H, Shirahashi N, and Kita T: Polymorphisms in four genes related to triglyceride and HDL-cholesterol levels in the general Japanese population in 2000. J Atheroscler Thromb, 2005; 12:240-250
15) Hallman DM, Boerwinkle E, Saha N, Sandholzer C, Menzel HJ, Csazar A, and Utermann G: The apolipoprotein E polymorphism: a comparison of allele frequencies and effects in nine populations. Am J Hum Genet, 1991; 49:338-349
16) Eto M, Watanabe K, and Ishii K: Reciprocal effects of apolipoprotein E alleles (epsilon 2 and epsilon 4) on plasma lipid levels in normolipidemic subjects. Clin Genet, 1986; 29:477-484
17) Kobori S, Nakamura N, Uzawa H, and Shichiri M: Influence of apolipoprotein E polymorphism on plasma lipid and apolipoprotein levels, and clinical characteristics of type Ⅲ hyperlipoproteinemia due to apolipoprotein E phenotype E2/2 in Japan. Atherosclerosis, 1988; 69:81-88

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Inhibition of MCP-1/CCR2 pathway ameliorates the development of diabetic nephropathy

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Abstract

Monocyte chemoattractant protein (MCP-1) is an important mediator for macrophage recruitment in atherosclerosis and various glomerulonephritis. However, the role of MCP-1 and its receptor CCR2 in the progression of diabetic nephropathy remains unknown. Using a type 1 diabetic nephropathy model that shows noticeable glomerulosclerosis, we examined the role of MCP-1/CCR2 by propagermanium (Pro; CCR2 antagonist) treatment, and confirmed it by transfection of plasmids carrying the 7ND (a mutant of MCP-1) gene. We measured the mesangial matrix expansion, type IV collagen (Col4), transforming growth factor (TGF)-β_1 positive area, and macrophage infiltration in glomeruli after 12 weeks. Mesangial matrix expansion and macrophage infiltration were increased in diabetic mice and inhibited by Pro or 7ND-treatment. Increased glomerular expression of Col4 and TGF-β_1 in diabetic mice was also ameliorated. Thus blocking the MCP-1/CCR2 pathway ameliorated glomerulosclerosis, indicating that the MCP-1/CCR2 pathway plays a crucial role in the progression of diabetic nephropathy.

Keywords: Diabetic nephropathy; Glomerulosclerosis; Macrophage; CCR2; Propagermanium

Diabetic nephropathy is the most common cause of end stage renal disease. Glomerulosclerosis is defined as segmental or global collapse or closure of capillary loops with associated mesangial extracellular matrix accumulation, and is the most important structural lesion of advanced diabetic nephropathy [1].

Recently, diabetic nephropathy has been considered as an inflammatory disease [2], in which macrophage infiltration into glomeruli is associated with the progression of glomerular injury [3]. For the macrophage infiltration MCP-1 plays a central role through its receptor, CCR2 [4,5]. In diabetic nephropathy, MCP-1 expression is increased in animal models [6], and urinary excretion of MCP-1 correlates not only with renal interstitial disturbance, but also with glomerular damage [7,8]. However, the role of MCP-1/CCR2 in the progression in diabetic nephropathy has not been fully elucidated.

We therefore asked whether the MCP-1/CCR2 pathway can contribute to diabetic glomerulosclerosis via macrophage infiltration. To address this question, we examined the effect of propagermanium (CCR2 antagonist) on diabetic glomerulosclerosis in transgenic mice (iNOS-Tg) overexpressing type 2 nitric-oxide synthase, which show prominent glomerulosclerosis [9,10]. To confirm the effect of propagermanium (Pro), we also utilized gene transfer

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of the 7ND gene, a deletion mutant of human MCP-1 (missing the N-terminal amino acids 2 through 8). Pro is shown to inhibit MCP-1-mediated chemotaxis [11] and its effect is already documented in macrophage-mediated inflammatory diseases [12]. 7ND is shown to work as a dominant-negative inhibitor of MCP-1 [13].

Here we report that blocking MCP-1/CCR2 signaling reduced macrophage infiltration and successfully inhibited the progression of diabetic glomerulosclerosis. Blockade of this signaling by Pro or the mutant gene delivery strategy could be a novel therapeutic approach for the treatment for diabetic nephropathy.

Methods

Experimental animals. iNOS-Tg mice were maintained on CD1/ICR background [14]. Male littermates were screened for the transgene by PCR amplification [14] and by blood glucose level using one touch ultra (Johnson & Johnson). All animal experiments were performed in accordance with institutional guidelines, and the Review Board of Kyoto University Granted Ethical permission to this study.

Protocol of treatment with Pro or 7ND. Four-week-old male iNOS-Tg mice and age-matched male CD1/ICR mice were used. At the age of 16 weeks, the mice were sacrificed.

Protocol 1: Pro administration. Mice were fed with diet supplemented with 0.005% (w/w) Pro (kindly provided by Sanwa Kagaku). The mice were divided into four groups; wild-type mice fed control diet (WT-C), wild-type mice fed diet with Pro (WT-Pro), diabetic mice fed control diet (DM-C), diabetic mice fed diet with Pro (DM-Pro).

Protocol 2: 7ND administration. The 7ND cDNA was a generous gift from Ken-ichi Nishida (Daiichi Pharmaceuticals). We injected into femoral muscle 100 μg plasmid carrying the 7ND cDNA or empty plasmid every two weeks as previously reported [15]. The mice were divided into four groups; wild-type mice treated with empty vector (WT-Sham), wild-type mice treated with 7ND (WT-7ND), diabetic mice treated with empty vector (DM-Sham), diabetic mice treated with 7ND (DM-7ND).

Histological examination. Kidney tissue blocks for light microscopy examination were fixed with Carnoy's solution and embedded in paraffin. Sections (2 μm) were stained with PASM. Kidney tissues were also snap frozen as previously described [16]. To study Col4 expression, cryostat sections (4 μm) were incubated with anti-Col4 antibody (1:250) (PROGEN), and then with FITC-labeled antibodies (1:100) (Chemicon). For the quantitation of TGF-β_1 expression, anti-TGF-β_1 antibody (1:50) (Santa Cruz) was used. Cryostat sections were first incubated with anti-TGF-β_1 antibody followed by anti-desmin antibody (1:100) (DAKO). TGF-β_1 was visualized with FITC-labeled antibodies, while desmin was visualized with Alexa Fluor 546-labeled antibodies (1:200) (Molecular Probes). For staining of macrophages, paraffin sections (5 μm) were pretreated with 10 mM sodium citrate buffer (pH 6.4) at 121 °C for 10 min, treated with 0.3% H_2O_2 in methanol, and then incubated with Avidin D and Biotin blocking solutions (VECTOR). Sections were then incubated with a biotin-labeled antibody for F4/80 (1:500) (BMA Biochemicals) or CD68 (1:200) (AbD serotec), using Tyramide Signal Amplification system according to manufacture's instructions (PerkinElmer Life Sciences). Diaminobenzidine tetrahydrochloride (DAB) staining followed by counterstaining with hematoxylin was performed.

Quantitation of light microscopy, macrophages and Col4 or TGF-β_1 expression in glomeruli. To assess glomerulosclerosis and glomerular hypertrophy, we measured the fraction of PASM-positive area (mesangial matrix fraction) and surface area in glomeruli [16]. Macrophages infiltrated in glomeruli were evaluated in DAB-treated tissues. The mean values of DAB-localized cells per glomerulus were calculated. Glomerular Col4 or TGF-β_1 expression was evaluated in FITC-labeled tissues as previously described [16]. For each animal, 50 glomeruli were analyzed.

Isolation of glomeruli by Dynabeads perfusion and quantitative PCR for MCP-1. Mice were perfused with 4.5 μm-diameter Dynabeads (Dynal A.S.), and then glomeruli were isolated [17]. After total RNA was isolated, cDNA synthesis was performed with SuperScript First-Strand Synthesis System (Invitrogen). Real-time PCR was performed using the ABI Prism 7700 sequence detection system (Applied Biosystems (AB)). TaqMan probes and primers for mouse ribosomal RNA and MCP-1 were used (Cat.4308329, ID:Mm00441242_m1; AB). PCR conditions were according to manufacture's instructions (Cat.4308329).

Measurment of MCP-1 and 7ND in serum. Plasma concentration of mouse MCP-1 and 7ND were measured as previously reported [15].

Statistical analysis. The data were analyzed using StatView (ver. 5). The data are expressed as the means ± SD. ANOVA with Bonferroni-Dunn test was used to identify significances in multiple comparisons between two groups. A level of $p < 0.05$ was considered statistically significant.

Results

Expression of MCP-1

We first isolated mRNA from glomeruli of diabetic and wild-type mice at 16 weeks retrieved by Dynabeads perfusion and quantitated MCP-1 expression by real-time PCR. A remarkable increase in the expression of MCP-1 mRNA in glomeruli was found in diabetic mice by the ratio of MCP-1/ribosomal RNA (DM; 2.12 ± 1.10 vs WT; 1.00 ± 0.23, $p = 0.038$).

Characteristics of four groups after Pro treatment

Diabetic mice showed a higher level of blood glucose and body weight-corrected kidney weight than in wild-type mice (Table 1).

Table 1
Characteristics of four groups of mice in protocol 1

	WT-C ($n = 6$)	WT-Pro ($n = 8$)	DM-C ($n = 9$)	DM-Pro ($n = 9$)
Blood glucose (mmol/l)	7.9 ± 1.6	9.0 ± 1.4	34.4 ± 1.8*	33.8 ± 3.4*
Blood pressure (mmHg)	116 ± 5	ND	114 ± 9	119 ± 8
Body weight (Bw) (g)	41.5 ± 1.3	44.6 ± 4.0	41.7 ± 2.4	39.3 ± 2.6*
Kidney weight/100 gBw (g)	0.87 ± 0.08	0.83 ± 0.09	1.10 ± 0.12*	1.15 ± 0.15*

The mice were separated into wild-type and diabetic groups with or without propagermanium treatment. Mice with propagermanium treatment (Pro) were administered 0.005% propagermanium-supplemented diet. After 12 weeks of propagermanium treatment (at the age of 16 weeks), systolic blood pressure was measured by the cuff-tailed method, and the mice were weighed (Bw) and sacrificed. Blood was taken to evaluate blood glucose. Kidneys were also taken to be weighed and corrected with Bw. The data are shown by means ± SD. *$p < 0.05$ vs WT, ND: Not done.

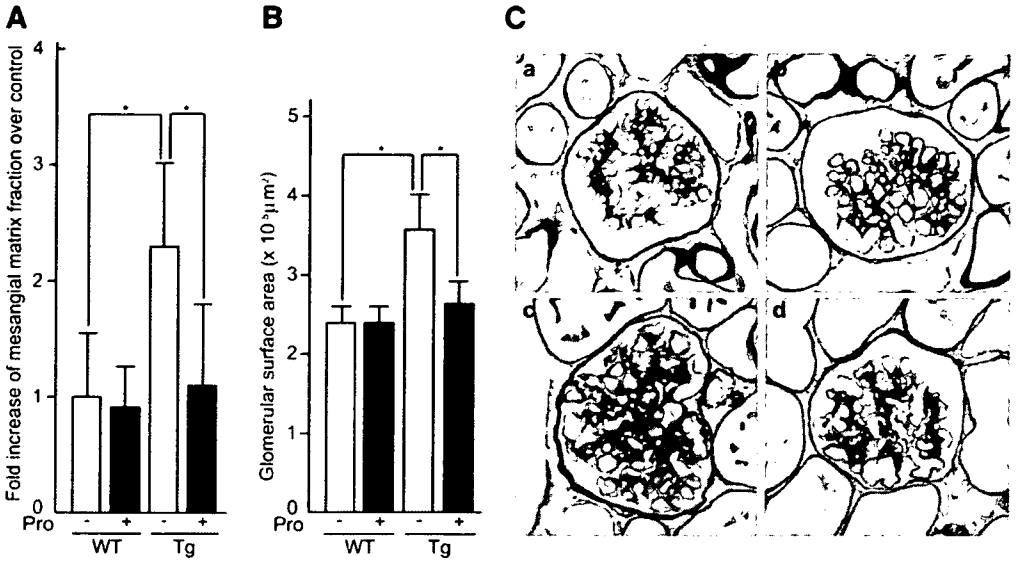

Fig. 1. Mesangial matrix fraction and glomerular surface area in four groups in protocol 1. Glomerular surface area and PASM-positive area were determined as described in methods. The mesangial matrix fraction was determined as percentage of PASM-positive area per total glomerular surface area, and then indicated as fold increase over control (WT-C). Randomly selected 50 glomeruli were examined in each mouse. Results are means ± SD. (A) The mesangial matrix fraction is shown. $^{*}p < 0.01$. (B) The Glomerular surface area is shown. $^{*}p < 0.01$. (C) Representative light microscopic pictures of glomeruli (PASM staining, 400× original magnification) from wild-type mice fed control diet (a), wild-type mice with propagermanium (b), diabetic mice fed control diet (c), diabetic mice with propagermanium (d).

Pro treatment ameliorates glomerulosclerosis, glomerular hypertrophy, and macrophage infiltration in glomeruli

DM-C showed 2.30-fold increases in glomerulosclerosis compared with WT-C, and its increase was inhibited by 52% in DM-Pro. DM-C also demonstrated 1.50-fold increases in glomerular area, and its increase was inhibited by 26% in DM-Pro (Fig. 1A–C). In DM-C, more F4/80-positive cells infiltrated into glomeruli were found than in WT-C (2.83 vs 0.260), and the number was reduced by 86% in DM-Pro (Fig. 2A and C). The number of CD68-positive cells in DM-C was also increased compared with that in WT-C (3.30 vs 0.169), and its number was also reduced by 87% in DM-Pro (Fig. 2B).

Pro treatment ameliorates Col4 expression as morphological changes in diabetic glomerulosclerosis

DM-C demonstrated 2.29-fold increases Col4 expression in glomerulus, and its increase was inhibited by 49% in DM-Pro (Fig. 3A). Col4 expression was mostly localized at the mesangial area by immunohistochemical analysis (Fig. 3B).

Pro treatment reduces TGF-β_1 expression in glomeruli

In DM-C, glomerular TGF-β_1 expression was approximately 3-times more than that in WT-C, and its expression was inhibited by 70% in DM-Pro (Fig. 4A). Expression of TGF-β_1 in DM-C seemed to be localized in mesangial areas, endothelium, and epithelium (Fig. 4B-a). Dual staining demonatrated that part of TGF-β_1-positive areas were also positive for desmin (Fig. 4B-c).

7ND treatment ameliorates glomerulosclerosis and macrophage infiltration in glomeruli

Table 2 shows the characteristics of four groups after 7ND treatment. The serum concentration of 7ND was highest at 1 week and became undetectable after 2 weeks, whereas MCP-1 was undetectable at any time point (Table 3). Glomerulosclerosis and glomerular hypertrophy were increased by 2.21-fold and 1.63-fold in DM-Sham compared with WT-Sham, respectively. These increases were ameliorated by 46% and 24% in DM-7ND, respectively (Supplementary Fig. 1). The number of glomerular infiltrated F4/80- or CD68-positive cells was also increased in DM-Sham compared with WT-Sham, and the number was reduced in DM-7ND by 83% (Supplementary Figs. 2A and C) or 89% (Supplementary Fig. 2B), respectively. It is worth mentioning that there was no noticeable systemic side effect in this procedure.

Discussion

In this study we demonstrated that blocking the MCP-1/CCR2 pathway ameliorates glomerulosclerosis through the inhibition of macrophage infiltration. Our study implies that the MCP-1/CCR2 pathway plays a crucial role in the progression of diabetic nephropathy.

It has recently been reported that the MCP-1-mediated pathway and/or macrophage infiltration play an important

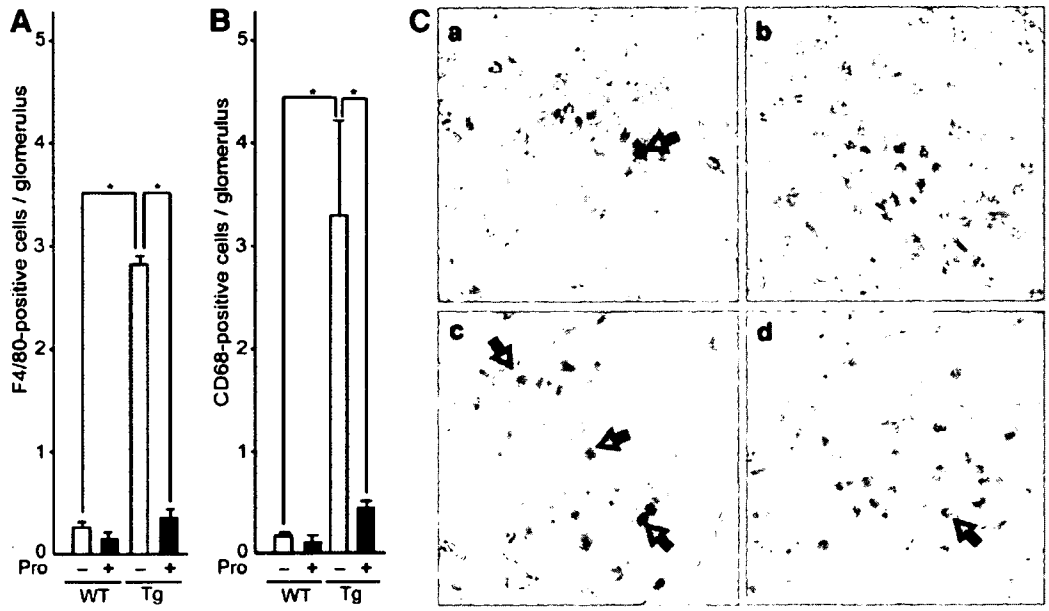

Fig. 2. F4/80- or CD68-positive cells in four groups in protocol 1. (A) The number of F4/80-positive cells was counted in randomly selected high-power fields (400×) of 50 glomeruli in each mouse. Results are means ± SD. $^*p < 0.01$. (B) The number of CD68-positive cells was counted in randomly selected high-power fields (400×) of 50 glomeruli in each mouse. Results are means ± SD. $^*p < 0.01$. (C) Representative light microscopic pictures (F4/80 staining) of glomeruli from wild-type mice fed control diet (a), wild-type mice with propagermanium (b), diabetic mice fed control diet (c), diabetic mice with propagermanium (d). F4/80-positive monocyte-macrophage is indicated with brown area (arrows).

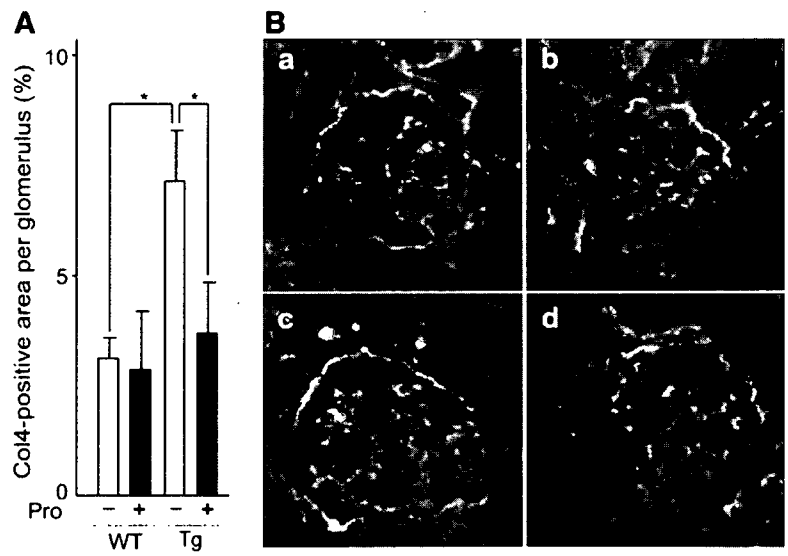

Fig. 3. Glomerular expression of Col4 in four groups in protocol 1. Glomerular surface area and FITC-positive area were determined as described in methods. Col4-positive fraction was determined as percentage of FITC-positive area per total glomerular surface area. Randomly selected 50 glomeruli were examined in each mouse. Results are means ± SD. (A) The Col4-positive fraction is shown. $^*p < 0.01$. (B) Representative immunohistochemistry (Col4 staining) of the glomeruli from wild-type mice fed control diet (a), wild-type mice with propagermanium (b), diabetic mice fed control diet (c), diabetic mice with propagermanium (d). The original magnification is 400×.

role in diabetic nephropathy. However, it remains to be determined that MCP-1 could be a target for the prevention of diabetic nephropathy. Moreover, no study has reported whether CCR2 could contribute to the progression of diabetic nephropathy.

To inhibit MCP-1/CCR2 signaling, we employed two distinct approaches. First, we fed mice with diet supplemented with Pro, a CCR2 antagonist. It is reported that by preventing the infiltration of macrophages, Pro has beneficial effects on inflammatory diseases [12]. It is conceivable that the main role of Pro is to inhibit macrophage infiltration through the CCR2-mediated pathway [11]. Chemokine receptors have redundancies for their ligands. For example, MCP-2, MCP-3, MCP-4, and MCP-5 are

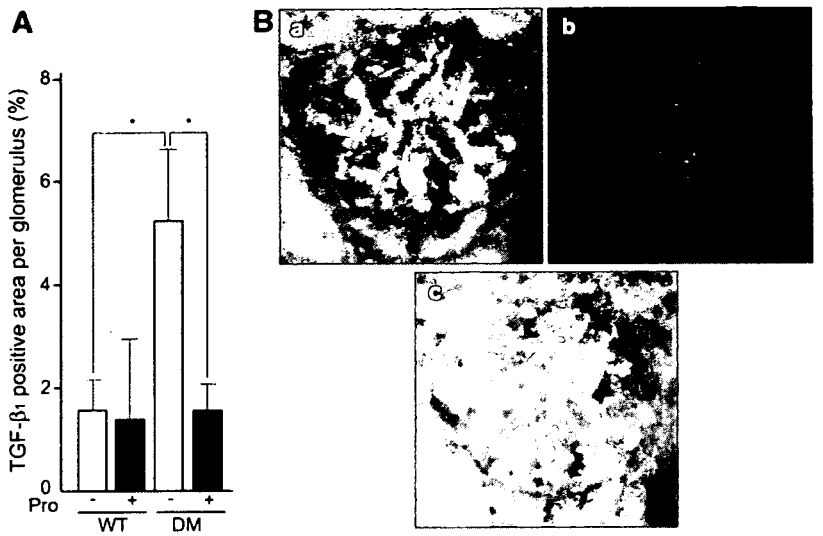

Fig. 4. Glomerular expression of TGF-β_1 in protocol 1. Glomerular surface area and FITC-positive area were determined as described in methods. The TGF-β_1-positive fraction was determined as percentage of FITC-positive area per total glomerular surface area. Randomly selected 50 glomeruli were examined in each mouse. Results are means ± SD. (A) The TGF-β_1-positive fraction is shown. $^*p < 0.01$. (B) Representative immunohistochemistry of glomeruli in untreated diabetic mice. (a) TGF-β_1-positive areas visualized with FITC, (b) desmin-positive areas with Alexa Fluor 546, and (c) overlay of TGF-β_1- and desmin-positive area were visualized. The original magnification is 400×.

Table 2
Characteristics of four groups of mice in protocol 2

	WT-Sham ($n = 7$)	WT-7ND ($n = 7$)	DM-Sham ($n = 5$)	DM-7ND ($n = 7$)
Blood glucose (mmol/l)	9.6 ± 2.6	11.1 ± 1.6	30.7 ± 8.4*	34.8 ± 2.4*
Blood pressure (mmHg)	110 ± 11	119 ± 12	119 ± 13	117 ± 9
Body weight (Bw) (g)	42.8 ± 3.4	45.1 ± 4.1	45.9 ± 2.8	41.3 ± 5.1
Kidney weight/100 gBw (g)	0.86 ± 0.12	0.73 ± 0.06	1.30 ± 0.13*	1.29 ± 0.21*

The mice were separated into wild-type and diabetic groups with or without 7ND treatment. Mice with 7ND treatment (7ND) were electroporated with 100 μg carrying the 7ND gene into the femoral muscle. After 12 weeks of 7ND treatment (at the age of 16 weeks), systolic blood pressure was measured by the cuff-tailed method, and the mice were weighed (Bw) and sacrificed. Blood was taken to evaluate blood glucose. Kidneys were also taken to be weighed and corrected with Bw. The data are shown by means ± SD.

* $p < 0.05$ vs WT.

Table 3
Expression of MCP-1 and 7ND after 7ND tansfection

	Baseline	No. of days after 7ND transfection		
		7	14	21
MCP-1 (pg/ml)	<9.0	<9.0	<9.0	<9.0
7ND (pg/ml)	<20	331 ± 255	<20	<20

The values are mean ± SD, $n = 12$. Serum 7ND concentrations were measured by the use of human MCP-1 ELISA kit (Biosource). Serum MCP-1 concentration were measured by murine MCP-1 ELISA kit (Biosource). Wild-type mice (WT) and diabetic mice (DM) were electroporated with 100 μg of plasmid DNA (pcDNA3-7ND) into the thigh muscle. The data of WT and DM are presented. The presented concentrations are below detectable limits except that of 7ND at day7.

also the ligands for CCR2 [18,19]. Therefore, the influence by MCP-2, MCP-3, MCP-4, and MCP-5 still remains a possibility. However, we achieved almost the same effect using another method; a gene delivery of dominant-negative inhibitor of MCP-1 (7ND). Therefore, in our study the impairment of glomerular lesions is thought to be due to the reduced infiltration of macrophage via the inhibition of the MCP-1/CCR2-mediated pathway.

In our study, diabetic glomerulosclerosis was ameliorated along with the inhibition of macrophage infiltration. Accumulating evidence suggests that in diabetic nephropathy, glomerulosclerosis is associated with TGF-β_1 expression [20,21], which is related to macrophage infiltration in glomeruli [22]. We performed TGF-β_1- and desmin-dual staining, which indicates that TGF-β_1 was expressed coincidence with mesangial areas to some extent. In this study, we demonstrated that TGF-β_1 expression was comparable with the severity of macrophage infiltration and that the inhibition of macrophage recruitment to the glomeruli resulted in a decrease of TGF-β_1 production. Thus, TGF-β_1 might be implicated in the diabetic glomerulosclerosis through macrophage infiltration.

In conclusion, we report here that the MCP-1/CCR2 pathway plays a crucial role in the progression of diabetic glomerulosclerosis, and suggest that blockade of this signaling by Pro or the mutant gene delivery approach

could be a novel therapeutic strategy for the treatment of the progression in diabetic nephropathy.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2007.06.148.

References

[1] A.B. Fogo, Mesangial matrix modulation and glomerulosclerosis, Exp. Nephrol. 7 (1999) 147–159.
[2] E. Galkina, K. Ley, Leukocyte recruitment and vascular injury in diabetic nephropathy, J. Am. Soc. Nephrol. 17 (2006) 368–377.
[3] T. Furuta, T. Saito, T. Ootaka, J. Soma, K. Obara, K. Abe, K. Yoshinaga, The role of macrophages in diabetic glomerulosclerosis, Am. J. Kidney Dis. 21 (1993) 480–485.
[4] L. Boring, J. Gosling, M. Cleary, I.F. Charo, Decreased lesion formation in CCR2−/− mice reveals a role for chemokines in the initiation of atherosclerosis, Nature 394 (1998) 894–897.
[5] W. Peters, I.F. Charo, Involvement of chemokine receptor 2 and its ligand, monocyte chemoattractant protein-1, in the development of atherosclerosis: lessons from knockout mice, Curr. Opin. Lipidol. 12 (2001) 175–180.
[6] S. Kato, V.A. Luyckx, M. Ots, K.W. Lee, F. Ziai, J.L. Troy, B.M. Brenner, H.S. MacKenzie, Renin-angiotensin blockade lowers MCP-1 expression in diabetic rats, Kidney Int. 56 (1999) 1037–1048.
[7] N. Banba, T. Nakamura, M. Matsumura, H. Kuroda, Y. Hattori, K. Kasai, Possible relationship of monocyte chemoattractant protein-1 with diabetic nephropathy, Kidney Int. 58 (2000) 684–690.
[8] H. Nishioka, M. Kanauchi, K. Dohi, Role of monocyte chemotactic peptide 1 in diabetic nephropathy, Nephron 88 (2001) 189–190.
[9] S. Ohashi, H. Abe, T. Takahashi, Y. Yamamoto, M. Takeuchi, H. Arai, K. Nagata, T. Kita, H. Okamoto, H. Yamamoto, T. Doi, Advanced glycation end products increase collagen-specific chaperone protein in mouse diabetic nephropathy, J. Biol. Chem. 279 (2004) 19816–19823.
[10] Y. Yamamoto, I. Kato, T. Doi, H. Yonekura, S. Ohashi, M. Takeuchi, T. Watanabe, S. Yamagishi, S. Sakurai, S. Takasawa, H. Okamoto, H. Yamamoto, Development and prevention of advanced diabetic nephropathy in RAGE-overexpressing mice, J. Clin. Invest. 108 (2001) 261–268.
[11] S. Yokochi, H. Hashimoto, Y. Ishiwata, H. Shimokawa, M. Haino, Y. Terashima, K. Matsushima, An anti-inflammatory drug, propagermanium, may target GPI-anchored proteins associated with an MCP-1 receptor, CCR2, J. Interferon. Cytokine Res. 21 (2001) 389–398.
[12] H. Shimokawa, Y. Eto, K. Miyata, K. Morishige, T. Kandabashi, K. Matsushima, A. Takeshita, Propagermanium suppresses macrophage-mediated formation of coronary arteriosclerotic lesions in pigs in vivo, J. Cardiovasc. Pharmacol. 41 (2003) 372–380.
[13] Y. Zhang, B.J. Rollins, A dominant negative inhibitor indicates that monocyte chemoattractant protein 1 functions as a dimer, Mol. Cell Biol. 15 (1995) 4851–4855.
[14] T. Takamura, I. Kato, N. Kimura, T. Nakazawa, H. Yonekura, S. Takasawa, H. Okamoto, Transgenic mice overexpressing type 2 nitric-oxide synthase in pancreatic beta cells develop insulin-dependent diabetes without insulitis, J. Biol. Chem. 273 (1998) 2493–2496.
[15] S. Inoue, K. Egashira, W. Ni, S. Kitamoto, M. Usui, K. Otani, M. Ishibashi, K. Hiasa, K. Nishida, A. Takeshita, Anti-monocyte chemoattractant protein-1 gene therapy limits progression and destabilization of established atherosclerosis in apolipoprotein E-knockout mice, Circulation 106 (2002) 2700–2706.
[16] T. Matsubara, H. Abe, H. Arai, K. Nagai, A. Mima, H. Kanamori, E. Sumi, T. Takahashi, M. Matsuura, N. Iehara, A. Fukatsu, T. Kita, T. Doi, Expression of Smad1 is directly associated with mesangial matrix expansion in rat diabetic nephropathy, Lab. Invest. 86 (2006) 357–368.
[17] M. Takemoto, N. Asker, H. Gerhardt, A. Lundkvist, B.R. Johansson, Y. Saito, C. Betsholtz, A new method for large scale isolation of kidney glomeruli from mice, Am. J. Pathol. 161 (2002) 799–805.
[18] M. Baggiolini, Chemokines in pathology and medicine, J. Intern. Med. 250 (2001) 91–104.
[19] M.N. Sarafi, E.A. Garcia-Zepeda, J.A. MacLean, I.F. Charo, A.D. Luster, Murine monocyte chemoattractant protein (MCP)-5: a novel CC chemokine that is a structural and functional homologue of human MCP-1, J. Exp. Med. 185 (1997) 99–109.
[20] S. Chen, B. Jim, F.N. Ziyadeh, Diabetic nephropathy and transforming growth factor-beta: transforming our view of glomerulosclerosis and fibrosis build-up, Semin. Nephrol. 23 (2003) 532–543.
[21] F.N. Ziyadeh, B.B. Hoffman, D.C. Han, M.C. Iglesias-De La Cruz, S.W. Hong, M. Isono, S. Chen, T.A. McGowan, K. Sharma, Long-term prevention of renal insufficiency, excess matrix gene expression, and glomerular mesangial matrix expansion by treatment with monoclonal antitransforming growth factor-beta antibody in db/db diabetic mice, Proc. Natl. Acad. Sci. USA 97 (2000) 8015–8020.
[22] M.P. Cohen, E. Shea, S. Chen, C.W. Shearman, Glycated albumin increases oxidative stress, activates NF-kappa B and extracellular signal-regulated kinase (ERK), and stimulates ERK-dependent transforming growth factor-beta 1 production in macrophage RAW cells, J. Lab. Clin. Med. 141 (2003) 242–249.

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Clinical research

Reassessment of the cutoff values of waist circumference and visceral fat area for identifying Japanese subjects at risk for the metabolic syndrome

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ABSTRACT

In the new world-wide criteria for metabolic syndrome (MetS) by the International Diabetes Federation (IDF) in 2006, the Japanese is the only ethnicity in which the recommended waist circumference (WC) cutoff value is higher in women (≧90 cm) than in men (≧85 cm), and its validity appears to be controversial. We investigated the optimal cutoff points for the diagnosis of central obesity in Japanese men and women, using the receiver operating characteristic (ROC) curve analysis for both of WC and visceral fat area (VFA) in 1870 middle-aged Japanese. VFA was superior to WC and Body mass index (BMI) for discriminating the subjects with two or more nonadipose components of MetS. The optimal cutoff points of VFA and WC were 132.6 cm^2 and 89.8 cm for men and 91.5 cm^2 and 82.3 cm for women. The stratifications of MetS components more than 1.0 in average occurred more steeply by the accumulation of VFA in women than in men.

In conclusion, setting the cutoff points of WC and VFA lower values in women than in men for the definition of central obesity is needed to identify the subjects with MetS in Japanese, as in other Asian populations.

1. Introduction

The number of subjects with metabolic syndrome (MetS) has markedly increased in Japan as well as in Western countries [1–3]. Because subjects with MetS are at increased risk for type 2 diabetes [4] and cardiovascular disease (CVD) [5], there is an urgent need to establish an appropriate and sensitive screening system to identify these high risk individuals and to prevent an epidemic of this syndrome.

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In 2006, the International Diabetes Federation (IDF) announced a new world-wide definition of MetS that is expected to be used in clinical practice in any country, including Japan [6]. For MetS diagnosis, special emphasis has been placed on the central obesity, as assessed by waist circumference (WC) cutoff values specific for ethnicity and gender. Accumulating evidence argues that lower cutoff points for WC should be used for Asians than for Europeans, as the former is prone to obesity-related morbidity and mortality at lower BMI and/or shorter WC than the latter [7–11]. Thus, the report of the joint WHO/IASO/IOTF committee proposed WC values of 90 cm for men and 80 cm for women as Asian-specific cutoff points for central adiposity [12]. These values have been adopted as the criteria for South Asians and Chinese in the IDF consensus [6].

Among the Japanese in particular, cutoff values of WC ≧85 cm for men and ≧90 cm for women have been adopted [6,13]. This is the only ethnic group in which the recommended WC cutoff point is higher in women than in men [6] and its validity appears to be controversial. It seems to be based on the 2002 report from the Examination Committee of Criteria for 'Obesity Disease' in Japan, which stated that the optimal cutoff value for identifying individuals at risk for obesity-related disorders was a visceral fat area (VFA) of 100 cm^2 measured by computed tomography (CT) in a study that combined both sexes [14]. The WC corresponding to 100 cm^2 of VFA was then identified as 85 cm in men and 90 cm in women [14]. Indeed, in the recent declaration of the criteria, the IDF added the annotation that Asian values (male 90 cm; female 80 cm) should be used for Japanese populations as well until more data are available [6].

There is only a little information to ascertain what would be appropriate cutoff points for indicators of central obesity in Japanese men and women. Much shorter cutoff points of WC in women by Hara et al. [15] and smaller cutoff points of VFA in women by Miyawaki et al. [16] have been proposed as more appropriate to predict the presence of multiple risk factors than the currently accepted values. Comparable results have also been shown by other studies in Japanese-Brazilian [17] and in Korean [18].

To address this controversial point, in this study we attempted to reassess the cutoff points for the diagnosis of central obesity in Japanese men and women, using the receiver operating characteristic (ROC) curve analysis for both of WC and VFA. To the best of our knowledge, this is the largest study analyzing both of these indicators for central adiposity in relation to the presence of components of MetS.

2. Materials and methods

2.1. Study population

Hokuriku Central Hospital has a special department, as a health service sponsored by their mutual aid association, where employees at public schools can receive routine medical checkups. Among subjects who enrolled in a regular medical checkup in 2006, 1893 persons were included in this study. Of these subjects, 93% were teachers, 5% were retired teachers, and 2% were secretaries at schools. All subjects voluntarily chose to be examined by CT for VFA values. After exclusion of 23 participants for missing answers on questionnaires regarding their medical histories, complete data were obtained for 1870 Japanese adults (1061 men and 809 women), whose age and body mass index (BMI) were 50.9 ± 7.6 years and 24.3 ± 2.9 kg/m^2, respectively. Signed informed consent was obtained from all subjects and the hospital review board approved the study protocol.

2.2. Anthropometric measurements and laboratory assays

All evaluations were performed at the health check department of Hokuriku Central Hospital. Anthropometric measurements of individuals wearing light clothing and without shoes were conducted by well-trained nurses. BMI was calculated by dividing weight (kg) by height squared (m^2). WC was taken at the end of normal expiration, measuring the minimum circumference at the level of umbilicus to the nearest 0.5 cm. Single CT scans (Aquilion, Toshiba Medical Systems, Tokyo, Japan) were obtained in the supine position at the end of inspiration. VFA was determined using commercial software, Fat Scan version 3.0 (N2 System, Osaka, Japan). Blood pressure (BP) was measured by automatic device (Colin Model BP-203RVII, Colin, Tokyo, Japan) after at least 5 min of rest in the sitting position.

After an overnight 12 h fast, blood samples were drawn to measure the levels of plasma glucose (PG), triglyceride, and HDL cholesterol. PG was determined by the glucose oxidase method (Automatic Glucose Analyzer ADAMS Glucose GA-1160, Arkray, Kyoto) and triglyceride and HDL cholesterol by enzymatic analytical chemistry (Autoanalyzer BioMajesty JCA-BM1650, JEOL Ltd., Tokyo, Japan).

2.3. Definition of metabolic syndrome

The nonadipose components of MetS were defined using the criteria of Japanese Society of Internal Medicine (JIM) [13] as the presence of two or more of the following: (1) TG ≧150 mg/dL (1.7 mmol/L) and/or HDL cholesterol <40mg/dL (<1.03 mmol/L) for both of men and women, or taking lipid-lowering medications (2) systolic BP ≧130 mmHg, diastolic BP ≧85 mmHg, or receiving antihypertensive medications; and (3) fasting PG ≧110 mg/dL (6.1 mol/L) or treatment with oral hypoglycemic medications or insulin.

2.4. Statistical analysis

Statistical analyses were conducted using SPSS version 11.0 for Windows (SPSS Inc., Chicago, IL, USA). Continuous variables were expressed as means ± standard deviations, and discrete variables were expressed as proportions. The ROC curve analyses were performed to determine the appropriate cut points for VFA, WC, and BMI in identifying subjects with two or more nonadipose components of MetS. Comparison of the diagnostic abilities of the tests was performed using the areas under the curves (AUC) and the significance of the difference between two areas was assessed by the method described by Hanley and McNeil [19]. The values of VFA, BMI, and WC that resulted in maximizing the Youden index (sensitivity + specificity − 1) were defined as optimal. Youden index is an integrative indicator of sensitivity and specificity [20,21].

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3. Results

3.1. Subject characteristics

Subject characteristics and the prevalence of metabolic risk factors are shown in Table 1. We found 392 (36.9%) of 1061 men and 106 (13.1%) of 809 women had two or more nonadipose components of MetS according to the criteria of JIM. Both men and women with two or more components of MetS had higher VFA, WC, and BMI than those without MetS.

3.2. ROC curve analyses

Fig. 1 shows ROC curves of VFA, WC, and BMI in men (A) and in women (B) which were used to identify subjects with two or more nonadipose components of MetS. All three curves lay above the diagonal line. Table 2 presents AUC values used to distinguish subjects with two or more nonadipose components of MetS or subjects with each component. In both men and women, VFA showed greater AUC values than WC and BMI, suggesting that VFA was superior to WC and BMI for discrimination of the subjects. Values in men were 0.675 for VFA (0.642–0.708), 0.641 for WC (0.607–0.675), and 0.642 for BMI (0.607–0.676). Similarly, in women, values were 0.759 for VFA (0.711–0.806), 0.686 for WC (0.636–0.735), and 0.727 for BMI (0.677–0.776).

Table 3 shows sensitivity, specificity, positive and negative predictive values, and the Youden index for VFA, WC, and BMI to detect subjects with two or more components of MetS according to the JIM criteria. In men, optimal cutoff points were 132.6 cm^2 for VFA, 89.8 cm for WC, and 24.1 kg/m^2 for BMI. In women, optimal cutoff points were 91.5 cm^2 for VFA, 82.3 cm for WC, and 23.1 kg/m^2 for BMI.

3.3. The number of MetS components by VFA level

The mean number of MetS components increased in proportion to the increase in VFA. The average value exceeded 1.0 at 90 and 110 cm^2 in men and women, respectively, but it exceeded 1.5 at lower VFA levels in women (140 cm^2) than in men (150 cm^2) (Fig. 2).

3.4. The prevalence of MetS according to the cutoff points obtained from this study.

According to the criteria by IDF and JIM, 25.8% of men and 4.2% of women in our sample were classified as MetS. If we adopted the criteria obtained from ROC analysis in this study, WC ≧89.8 cm in men and WC ≧82.3 cm in women, the revised prevalence of MetS in our population was 16.5% in men and 9.4% in women.

4. Discussion

These cross-sectional data comprising 1870 middle-aged Japanese subjects demonstrated that the cutoff points of 132.6 cm^2 VFA, 89.8 cm WC, and 24.1 kg/m^2 BMI for men and

Table 1 – Characteristics of study subjects according to metabolic syndrome status in Japanese men and women

	Men			Women		
	Total	Two or more nonadipose components of the JIM criteria other than waist circumference		Total	Two or more nonadipose components of the JIM criteria other than waist circumference	
		Absent	Present		Absent	Present
n	1061	669	392	809	703	106
Age (years)	50.6 ± 7.8	50.2 ± 8.2	51.4 ± 7.2	51.2 ± 7.2	50.7 ± 7.4	53.9 ± 5.2
BMI (kg/m^2)	24.9 ± 2.7	24.4 ± 2.4	25.7 ± 2.8	23.5 ± 3.1	23.2 ± 2.9	25.7 ± 3.4
Waist circumference (cm)	86.9 ± 7.1	85.5 ± 6.7	89.2 ± 7.2	81.9 ± 8.2	81.2 ± 8.1	86.6 ± 7.5
VFA (cm^2)	128 [97, 159]	117 [90, 149]	148 [115, 178]	69 [48, 98]	66 [45.5, 92]	105 [73, 135]
Type 2 diabetes or fasting PG ≧110 mg/dl (%)	17.3	3.4	41.1	5.8	1.0	37.7
Taking oral hypoglycemic medication or insulin (%)	4.0	1.0	8.9	0.7	0.0	5.7
High blood pressure (%)[a]	60.4	40.4	94.6	42.5	34.0	99.1
Taking antihypertensive medications (%)	13.6	7.2	24.5	11.9	8.4	34.9
HDL cholesterol (mg/dl)	54.1 ± 12.4	55.8 ± 12.2	51.1 ± 12.3	64.2 ± 14.3	65.4 ± 13.9	56.6 ± 14.8
Low HDL cholesterol (%)[b]	9.3	5.2	16.3	1.9	0.6	10.4
Triglycerides (mg/dl)	122 [87, 172]	104 [75, 134]	172 [128,227]	85 [61, 118]	80 [59, 106]	153 [106, 182]
Hypertriglyceridemia (%)	35.2	15.7	68.6	11.2	4.7	54.7
Taking lipid-lowering medication (%)	7.4	1.9	16.6	6.9	3.8	27.4

Data are mean ± S.D., median [interquartile range], or %. JIM, Japanese Society of Internal Medicine; PG, plasma glucose; WC, waist circumference.

[a] High blood pressure was diagnosed if systolic blood pressure was ≧130 mmHg, diastolic blood pressure was ≧85 mmHg, or the subject was receiving antihypertensive medications.

[b] Low HDL cholesterol was diagnosed if HDL cholesterol was <40 mg/dl in men and women.

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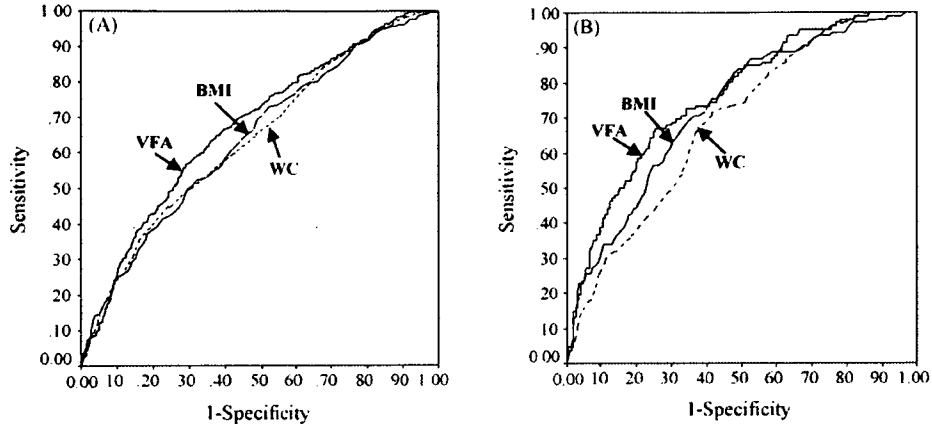

Fig. 1 – The ROC curves for visceral fat area (VFA), waist circumference (WC) and body mass index (BMI) to predict the presence of two or more components of MetS, as defined by the criteria by Japanese Society of Internal Medicine (JIM), in men (A) and in women (B).

91.5 cm^2 VFA, 82.3 cm WC, and 23.1 kg/m^2 BMI for women were optimal to yield maximal sensitivity plus specificity for predicting two or more nonadipose components of MetS. VFA was superior to WC and BMI in identifying subjects with multiple risk factors both in men and women. Given our findings, WC cutoff values for the Japanese population recommended by the IDF [6] and JIM [13] should be reevaluated, especially in women.

To date, there have been a few studies supporting a lower WC cutoff point than that currently accepted for Japanese women. Hara et al. analyzed data from a community-based cohort of 692 Japanese subjects and proposed 85 cm in men and 78 cm in women as optimal to identify subjects with multiple risk factors of MetS defined by National Cholesterol Education Program-Adult Treatment Panel (NCEP) III [15]. In another study, in terms of predicting the insulin resistance,

Table 2 – Areas under the ROC curve of WC, BMI, and VFA to identify the presence of components of MetS

	Men (n = 1061)		Women (n = 809)	
	ROC curve area (95% CI)	P value compared with WC[a]	ROC curve area (95% CI)	P value compared with WC[a]
Two or more nonadipose components of the JIM criteria other than waist circumference				
VFA	0.675 (0.642–0.708)	0.164	0.759 (0.711-0.806)	0.046
WC	0.641 (0.607–0.675)		0.686 (0.636–0.735)	
BMI	0.642 (0.607–0.676)	0.971	0.727 (0.677–0.776)	0.175
Fasting plasma glucose ≧110 mg/dl or type 2 diabetes				
VFA	0.612 (0.566–0.658)	0.072	0.760 (0.695–0.824)	0.352
WC	0.553 (0.508–0.598)		0.712 (0.634–0.790)	
BMI	0.549 (0.502–0.595)	0.951	0.740 (0.668–0.812)	0.604
High blood pressure[b]				
VFA	0.641 (0.607–0.676)	0.751	0.686 (0.649–0.723)	0.236
WC	0.634 (0.600–0.668)		0.654 (0.616–0.692)	
BMI	0.637 (0.603–0.671)	0.882	0.683 (0.646–0.720)	0.285
Hypertriglyceridemia				
VFA	0.664 (0.631–0.697)	0.192	0.748 (0.700–0.797)	0.026
WC	0.632 (0.597–0.666)		0.664 (0.609–0.720)	
BMI	0.637 (0.602–0.671)	0.855	0.663 (0.603–0.723)	0.967
Low HDL cholesterol[c]				
VFA	0.614 (0.557–0.670)	0.880	0.645 (0.571–0.718)	0.148
WC	0.625 (0.567–0.682)		0.570 (0.502–0.638)	
BMI	0.618 (0.561–0.674)	0.804	0.593 (0.526–0.661)	0.655

JIM, Japanese Society of Internal Medicine; WC, waist circumference; VFA, visceral fat area.

[a] P value compared with waist circumference by the method of Hanley and MnNeil.

[b] High blood pressure was diagnosed if systolic blood pressure was ≧130 mmHg, diastolic blood pressure was ≧85 mmHg, or the subject was receiving antihypertensive medications.

[c] Low HDL cholesterol was diagnosed if HDL cholesterol was <40 mg/dl in men and women.

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Table 3 – Sensitivity and specificity of VFA, WC, and BMI to detect subjects with two or more nonadipose components of the JIM criteria

	Cut point	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	*J* value[a]
Men (n = 1061)						
VFA (cm^2)						
Optimal cutoff point[b]	132.6	63.3	64.3	51.0	74.9	0.28
JIM cutoff point for Japanese	100	83.4	34.8	42.8	78.2	0.18
Waist circumference (cm)						
Optimal cutoff point[b]	89.8	44.6	76.1	52.2	70.1	0.21
JIM cutoff point for Japanese	85	69.9	45.1	42.7	71.9	0.15
IDF cutoff point for South Asians and Chinese	90	44.6	76.1	52.2	70.1	0.21
IDF cutoff point for Europids	94	26.0	88.3	56.6	67.1	0.14
BMI (kg/m^2)						
Optimal cutoff point[b]	24.1	73.0	48.0	45.1	75.2	0.21
Overweight	25	56.1	61.3	45.9	70.4	0.17
Obesity	30	7.7	97.8	67.2	64.4	0.05
Women (n = 809)						
VFA (cm^2)						
Optimal cutoff point[b]	91.5	67.0	74.4	28.3	93.7	0.41
JIM cutoff point for Japanese	100	51.9	81.4	29.6	91.8	0.33
Waist circumference (cm)						
Optimal cutoff point[b]	82.3	71.7	58.5	20.7	93.2	0.30
JIM cutoff point for Japanese	90	32.1	86.2	26.0	89.4	0.18
IDF cutoff point for South Asians, Chinese, and Europids	80	82.1	42.5	33.4	87.1	0.25
BMI (kg/m^2)						
Optimal cutoff point[b]	23.1	83.0	52.2	20.7	95.3	0.35
Overweight	25.0	50.0	77.2	24.8	91.1	0.27
Obesity	30.0	14.2	98.2	54.3	88.4	0.12

PPV, positive predictive value; NPV, negative predictive value; JIM, Japanese Society of Internal Medicine.

[a] J = sensitivity + specificity − 1.

[b] The optimal cut point was obtained from Youden index as [maximun (J = sensitivity + specificity − 1)].

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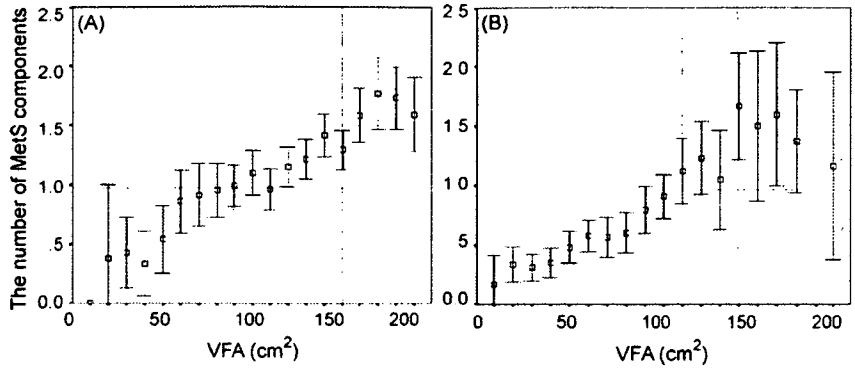

Fig. 2 – The mean number of nonadipose components of MetS by visceral fat area (VFA) in men (A) and in women (B). The dotted lines represent the levels at which an average number of components exceeded 1.0 and 1.5, respectively. The VFA value represents the level of VFA divided by each 10 cm^2.

the WC cutoff level was proposed to be 83 cm in men and 75 cm in women [22]. In these studies, they employed ROC curve analyses to identify cutoff points using Youden index (maximum [sensitivity + specificity − 1]), which was also employed in our study.

A recent study investigated the optimal cutoff points for both WC and VFA in 639 Japanese-Americans [23]. Consistent with our findings, they demonstrated that VFA was better than WC and BMI for identification of subjects with at least two nonadipose components of MetS and that the optimal cutoff points of VFA as well as WC were lower in women than in men: 96 cm^2 VFA and 90 cm WC for men, and 75 cm^2 VFA and 84 cm WC for women. Their WC cutoff values were comparable to those found in our data, but VFA cutoff points were larger in our study than theirs. It is not clear whether it was due to methodological differences in VFA measurement or to possible differences in the composition of body fat of Japanese-Americans from that of native Japanese. The percentage of subjects receiving treatment of diabetes was much higher in Japanese-Americans than in our population (45.8% of men and 44.9% of women vs. 4.0% of men and 0.7% of women). Differences in life-style may affect the cutoff points between these two populations who share a genetic background.

The prevalence of MetS according to the cutoff points obtained from this study would be a better reflection of the true prevalence of MetS because it is consistent with the mortality from coronary heart disease (35.7 male vs. 17.5 female, per 100,000) and cerebrovascular disease (60.5 male vs. 38.4 female, per 100,000) in Japan [24,25].

VFA was superior to WC and BMI in identifying subjects with two or more nonadipose components of MetS, as shown by the larger AUC of VFA. Indeed, it has been reported that insulin resistance is associated more strongly with VFA directly measured by CT than with waist-to-hip ratio or WC [26–28]. This would also apply to the presence of MetS components. Although WC is an inexpensive and practical screening tool for diagnosing central obesity and serves as a surrogate indicator for visceral fat, its inclusion of subcutaneous fat or skeletal muscle mass is problematic [29].

A VFA value of 100 cm^2 has been noted as the gold standard in defining the central obesity in Japanese men and women [13,14]. It is based on the report that the average number of obesity-related disorders exceeded 1.0 at the VFA value of 100 cm^2 in a study sample that combined both sexes [14]. As Hayashi et al. noted [23], this approach did not consider the possibility that the associations between the components of MetS and VFA might vary by sex, which was evident in our study as well as the MONK study [16] and a recent study in Japanese-Americans [23]. Moreover, according to the values of sensitivity and specificity for detecting subjects with multiple risk factors reported in that landmark study [14], Youden indexes were comparable at VFA values from 90 to 110 cm^2. Our process of assessing cut points for central obesity is more refined than that of the classical study [14] in two points; (1) the optimal cut points for VFA were analyzed in men and women separately. (2) Using ROC analysis, the balance of specificity and sensitivity for identifying subjects at risk for MetS was better than theirs. The optimal cutoff point for VFA in men that we proposed in the present study appears to be much higher than 100 cm^2. This might be due to the fact that our study sample was limited to the middle-aged Japanese men. Indeed, Hayashi et al. have reported that the cut points for VFA were different by generation [23]. So, further investigation is needed to broaden to apply this cut points to other generations.

A novel finding of our study is that the average number of MetS components exceeded 1.0 at smaller VFA values in men than in women but exceeded 1.5 at smaller VFA values in women than in men. This indicates that the stratification of MetS components in the range from 1.0 to 1.5 occurs more drastically by visceral fat accumulation in women than in men. On average, men had twice as much visceral fat as women and high prevalence of MetS. However, the accumulation of visceral fat may be more detrimental in women than in men. This is in line with other studies showing that relative risk of death from CVD is increased eight times in women with the highest waist-to-hip ratio [30] but only two times in men [31]. These gender differences suggest that it may be important to assess the relationships between VFA and risk factors in women separately from men.

There are several limitations to our study. First, the subjects were not randomly selected but limited to employees

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of public schools. However, the metabolic and anthropometric profiles of subjects in this study were largely in agreement with results from other studies [15,16], suggesting that this sample is representative of the working middle-aged population in Japan. Second, the cutoff points were investigated for all ages combined. The optimal cutoff points may differ by age-group [32,33] or BMI [34]. This relationship may also be more complex in postmenopausal women, who are predisposed to selective fat storage in the visceral region [35]. Finally, our analysis of this cross-sectional data cannot provide causal explanations. Further studies are needed to prospectively relate the accumulation of visceral fat to the presence of risk factors, or to the incidence of CVD.

In conclusion, our data indicate that the optimal cutoff points of VFA as well as WC for identifying subjects at risk for MetS differ between men and women. Setting the cutoff points of WC and VFA lower values in women than in men for the definition of central obesity should be more useful to identify the subjects with MetS in Japanese, as in other Asian populations.

Conflicts of interest

All authors do not have any financial and personal relationships with other people or organizations that could inappropriately influence (bias) their work, all within 3 years of beginning the work submitted. None to declare.

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REFERENCES

[1] H. Takeuchi, S. Saitoh, S. Takagi, H. Ohnishi, J. Ohhata, T. Isobe, et al., Metabolic syndrome and cardiac disease in Japanese men: applicability of the concept of metabolic syndrome defined by the National Cholesterol Education Program-Adult Treatment Panel III to Japanese men-the Tanno and Sobetsu Study, Hypertens. Res. 28 (2005) 203–208.

[2] H. Tanaka, T. Shimabukuro, M. Shimabukuro, High prevalence of metabolic syndrome among men in Okinawa, J. Atheroscler. Thromb. 12 (2005) 284–288.

[3] I. Saito, M. Mori, H. Shibata, H. Hirose, M. Tsujioka, H. Kawabe, Prevalence of metabolic syndrome in young men in Japan, J. Atheroscler. Thromb. 14 (2007) 27–30.

[4] S.M. Haffner, R.A. Valdez, H.P. Hazuda, B.D. Mitchell, P.A. Morales, M.P. Stern, Prospective analysis of the insulin-resistance syndrome (syndrome X), Diabetes 41 (1992) 715–722.

[5] H.M. Lakka, D.E. Laaksonen, T.A. Lakka, L.K. Niskanen, E. Kumpusalo, J. Tuomilehto, et al., The metabolic syndrome and total and cardiovascular disease mortality in middle-aged men, JAMA 288 (2002) 2709–2716.

[6] K.G. Alberti, P. Zimmet, J. Shaw, Related metabolic syndrome—a new world-wide definition. A consensus statement from the International Diabetes Federation, Diabet. Med. 23 (2006) 469–480.

[7] P.M. McKeigue, B. Shah, M.G. Marmot, Relation of central obesity and insulin resistance with high diabetes prevalence and cardiovascular risk in South Asians, Lancet 337 (1991) 382–386.

[8] G.T. Ko, J.C. Chan, C.S. Cockram, J. Woo, Prediction of hypertension, diabetes, dyslipidaemia or albuminuria using simple anthropometric indexes in Hong Kong Chinese, Int. J. Obes. Relat. Metab. Disord. 23 (1999) 1136–1142.

[9] A. Raji, E.W. Seely, R.A. Arky, D.C. Simonson, Body fat distribution and insulin resistance in healthy Asian Indians and Caucasians, J. Clin. Endocrinol. Metab. 86 (2001) 5366–5371.

[10] M. Deurenberg-Yap, S.K. Chew, V.F. Lin, B.Y. Tan, W.A. van Staveren, P. Deurenberg, Relationships between indices of obesity and its co-morbidities in multi-ethnic Singapore, Int. J. Obes. Relat. Metab. Disord. 25 (2001) 1554–1562.

[11] A. Misra, N.K. Vikram, R. Gupta, R.M. Pandey, J.S. Wasir, V.P. Gupta, Waist circumference cutoff points and action levels for Asian Indians for identification of abdominal obesity, Int. J. Obes. 30 (2006) 106–111.

[12] WHO Western Pacific Regional Office, IOTF, IASO, The Asia-Pacific perspective: redefining obesity and its treatment, In Health Communications Australia Sydney, 2000.

[13] Y. Matsuzawa, Metabolic syndrome-definition and diagnostic criteria in Japan, J. Jpn. Soc. Int. Med. 94 (2005) 188–203.

[14] Examination Committee of Criteria for 'Obesity Disease' in Japan, Japan Society for the Study of Obesity; New criteria for 'obesity disease' in Japan, Circ. J. 66 (2002) 987–992.

[15] K. Hara, Y. Matsushita, M. Horikoshi, N. Yoshiike, T. Yokoyama, H. Tanaka, et al., A proposal for the cutoff point of waist circumference for the diagnosis of metabolic syndrome in the Japanese population, Diabetes Care 29 (2006) 1123–1124.

[16] T. Miyawaki, M. Hirata, K. Moriyama, Y. Sasaki, H. Aono, N. Saito, et al., Metabolic syndrome in Japanese diagnosed with visceral fat measurement by computed tomography, Proc. Jpn. Acad. 81 (2005) 471–479.

[17] Ferreira SR for the Japanese-Brazilian Diabetes Study Group, Analysis of criteria for metabolic syndrome in a population-based study of Japanese-Brazilians, Diabetes Obes. Metab. 7 (2005) 352–359.

[18] S.Y. Lee, H.S. Park, D.J. Kim, J.H. Han, S.M. Kim, G.J. Cho, et al., Appropriate waist circumference cutoff points for central obesity in Korean adults, Diabetes Res. Clin. Pract. 75 (2007) 72–80.

[19] J.A. Hanley, B.J. McNeil, A method of comparing the areas under receiver operating characteristic curves derived from the same cases, Radiology 148 (1983) 839–843.

[20] W.J. Youden, An index for rating diagnostic tests, Cancer 3 (1950) 32–35.

[21] N.J. Perkins, E.F. Schisterman, The inconsistency of "optimal" cutpoints obtained using two criteria based on the receiver operating characteristic curve, Am. J. Epidemiol. 163 (2006) 670–675.

[22] T. Ohkubo, M. Kikuya, K. Asayama, Y. Imai, A proposal for the cutoff point of waist circumference for the diagnosis of metabolic syndrome in the Japanese population, Diabetes Care 29 (2006) 1986–1987.

[23] T. Hayashi, E.J. Boyko, M.J. McNeely, D.L. Leonetti, S.E. Kahn, W.Y. Fujimoto, Minimum waist and visceral fat values for identifying Japanese Americans at risk for the metabolic syndrome, Diabetes Care 30 (2007) 120–127.

[24] F. Levi, F. Lucchini, E. Negri, C. La Vecchia, Trends in mortality from cardiovascular and cerebrovascular

Please cite this article in press as: R. Oka et al., Reassessment of the cutoff values of waist circumference and visceral fat area for identifying Japanese subjects at risk for the metabolic syndrome, Diab. Res. Clin. Pract. (2007), doi:10.1016/j.diabres.2007.10.016

diseases in Europe and other areas of the world, Heart 88 (2002) 119–124.
[25] A. Okayama, H. Ueshima, M. Marmot, P. Elliott, S.R. Choudhury, Y. Kita, Generational and regional differences in trends of mortality from ischemic heart disease in Japan from 1969 to 1992, Am. J. Epidemiol. 153 (2001) 1191–1198.
[26] D.G. Carey, A.B. Jenkins, L.V. Campbell, J. Freund, D.J. Chisholm, Abdominal fat and insulin resistance in normal and overweight women: direct measurements reveal a strong relationship in subjects at both low and high risk of NIDDM, Diabetes 45 (1996) 633–638.
[27] M. Brochu, R.D. Starling, A. Tchernof, D.E. Matthews, E. Garcia-Rubi, E.T. Poehlman, Visceral adipose tissue is an independent correlate of glucose disposal in older obese postmenopausal women, J. Clin. Endocrinol. Metab. 85 (2000) 2378–2384.
[28] S. Lemieux, Contribution of visceral obesity to the insulin resistance syndrome, Can. J. Appl. Physiol. 26 (2001) 273–290.
[29] A. Misra, A. Garg, N. Abate, R.M. Peshock, J. Stray-Gundersen, S.M. Grundy, Relationship of anterior and posterior subcutaneous abdominal fat to insulin sensitivity in nondiabetic men, Obes. Res. 5 (1997) 93–99.
[30] L. Lapidus, C. Bengtsson, B. Larsson, K. Pennert, E. Rybo, L. Sjostrom, Distribution of adipose tissue and risk of cardiovascular disease and death: a 12 year follow up of participants in the population study of women in Gothenburg, BMJ 289 (1984) 1257–1261.
[31] B. Larsson, K. Svardsudd, L. Welin, L. Wilhelmsen, P. Bjorntorp, G. Tibblin, Abdominal adipose tissue distribution, obesity, and risk of cardiovascular disease and death: 13 year follow up of participants in the study of men born in 1913, BMJ 288 (1984) 1401–1404.
[32] E.S. Ford, W.H. Giles, W.H. Dietz, Prevalence of the metabolic syndrome among US adults: findings from the third National Health and Nutrition Examination Survey, JAMA 287 (2002) 356–359.
[33] C.E. Tan, S. Ma, D. Wai, S.K. Chew, E.S. Tai, Can we apply the National Cholesterol Education Program Adult Treatment Panel definition of the metabolic syndrome to Asians? Diabetes Care 27 (2004) 1182–1186.
[34] S. Zhu, Z. Wang, S. Heshka, M. Heo, M.S. Faith, S.B. Heymsfield, Waist circumference and obesity-associated risk factors among whites in the third National Health and Nutrition Examination Survey: clinical action thresholds, Am. J. Clin. Nutr. 76 (2002) 743–749.
[35] M.J. Toth, A. Tchernof, C.K. Sites, E.T. Poehlman, Menopause-related changes in body fat distribution, Ann. N.Y. Acad. Sci. 904 (2000) 502–506.

Please cite this article in press as: R. Oka et al., Reassessment of the cutoff values of waist circumference and visceral fat area for identifying Japanese subjects at risk for the metabolic syndrome, Diab. Res. Clin. Pract. (2007), doi:10.1016/j.diabres.2007.10.016

Circ J 2007; **71**: 1734–1737

Generation and Gender Differences in the Components Contributing to the Diagnosis of the Metabolic Syndrome According to the Japanese Criteria

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Background To clarify whether there are age and gender differences in the components contributing to the diagnosis of metabolic syndrome (MS) in the Japanese population.

Methods and Results A total of 21,870 individuals (ie, 7,329 men aged 68±8.1 years, body mass index (BMI) 23.2±2.9 kg/m^2 and 14,541 women aged 66±9.4 years, BMI 22.8±3.3 kg/m^2) participated in the study. The subjects were obtained from the general population and examinations were conducted in hospitals located in Kanazawa city. MS was diagnosed according to the Japanese criteria. Information regarding medication is lacking in all participating subjects. Overall, the incidence of MS was 18.4% and 5.78% for men and women, respectively. When analyzed according to age group, the incidence of MS in men did not differ significantly, whereas its prevalence was higher in older women than in younger women. Among the indicators of MS, high blood pressure (BP; high systolic BP and/or high diastolic BP) was the most frequent, followed by dyslipidemia (high triglycerides and/or low high-density lipoprotein-cholesterol (HDL-C)), and high fasting plasma glucose was the least frequently occurring in both genders. In contrast to the high frequency of high BP, isolated high diastolic BP was rare across both genders regardless of age group. Similarly, isolated low HDL-C was quite rare.

Conclusions Frequency of the components contributing to the diagnosis of MS differed considerably according to gender and age group in the Japanese population. (*Circ J* 2007; **71**: 1734–1737)

Key Words: Atherosclerosis, Carotid artery; Coronary artery; Triglycerides; Visceral fat

There is accumulating evidence that metabolic syndrome (MS) is highly related to the incidence of type 2 diabetes mellitus and cardiovascular disease[1–5] Insulin resistance or visceral fat accumulation is considered to be an important factor causing MS[6–9] However, the genetic background of subjects with MS appears to be quite heterogeneous. The frequency of MS in the general population has been reported in several populations using several types of modified criteria for the diagnosis of MS[10–16] To our knowledge, there have been few reports on age and gender differences among the components, which include high systolic blood pressure (SBP) and/or high diastolic blood pressure (DBP), high triglycerides (TG), low high-density lipoprotein-cholesterol (HDL-C) and high fasting plasma glucose (FPG), contributing to the diagnosis of MS.

Recently, it has been reported that among 8,144 Japanese subjects who underwent a routine medical checkup, 1,251 individuals were diagnosed with MS according to NCEP-ATPIII, with high blood pressure (BP) being the most common component of MS[14] In 2005, the published Japanese criteria for MS was defined as visceral obesity plus at least 2 of the following metabolic disorders: hyperglycemia (FPG ≥110 mg/dl), high blood pressure (SBP ≥130 and/or DBP ≥85 mmHg), dyslipidemia (high TG (TG ≥1.7 mmol/L) and/or low HDL-C (HDL-C <1.0 mmol/L))[17–19] The overall frequency of MS in the general Japanese population is estimated to be 12.1% in men and 1.7% in women, according to this criteria[13] As mentioned above, each component of MS does not appear to contribute equally to the diagnosis

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Table 1 Clinical Profile of All Study Subjects

	Men (n=7,329)	*Women (n=14,541)*
Age (years)	*68.1±8.1*	*66.1±9.37*
BMI (kg/m^2)	*23.2±2.9*	*22.8±3.29*
Waist circumference (cm)	*82.7±9.6*	*84.5±8.2*
SBP (mmHg)	*131±16.9*	*129±17.4*
DBP (mmHg)	*78.3±10.6*	*76.0±10.3*
FPG (mmo/L)	*5.63±1.44*	*5.21±1.06*
HbA$_{1c}$ (%)	*5.7±0.9*	*5.5±0.7*
TC (mmo/L)	*5.06±0.85*	*5.51±0.83*
HDL-C (mmo/L)	*1.48±0.40*	*1.74±0.42*
TG (mmo/L)	*1.16 (0.85, 1.67)*	*1.04 (0.78, 1.41)*
LDL-C (mmo/L)	*2.94±0.79*	*3.22±0.78*
UA (mmo/L)	*0.35±0.08*	*0.28±0.07*
Creatinine (mmo/L)	*0.07±0.03*	*0.05±0.01*

Values are shown as the mean±SD except for TG, which are shown as the median (1st, 3rd quartile). The survey was conducted from May 1, 2005 through to October 31, 2005.
BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting plasma glucose; Hb, hemoglobin; TC, total cholesterol; HDL-C, high-density lipoprotein-cholesterol; TG, triglycerides; LDL-C, low-density lipoprotein-cholesterol; UA, uric acid.

Table 2 Clinical Profile of the Subjects With or Without MS

	MS		Non-MS	
	Men (n=1,348)	Women (n=840)	Men (n=5,981)	Women (n=13,701)
Age (years)	68.0±7.92	69.5±8.60**	68.1±8.2	65.9±9.4
BMI (kg/m^2)	25.4±2.36**	27.1±3.17**	22.7±2.7	22.5±3.1
Waist circumference (cm)	91.8±5.41**	96.6±5.77**	82.9±7.8	80.9±9.7
SBP (mmHg)	141±13.9**	142±13.2**	129±16.7	128±17.2
DBP (mmHg)	83.2±10.0**	81.9±9.66**	77±10.4	75.6±10.2
FPG (mmol/L)	6.52±1.87**	6.30±1.63**	5.39±1.23	5.12±0.98
HbA1c (%)	6.14±1.16**	6.13±1.07**	5.6±0.8	5.5±0.7
TC (mmol/L)	5.22±0.93**	5.64±0.88**	5.02±0.82	5.5±0.82
HDL-C (mmol/L)	1.27±0.32**	1.46±0.34**	1.53±0.4	1.75±0.41
TG (mmol/L)	1.90 (1.44, 2.44)**	1.84 (1.31, 2.26)**	1.07 (0.8, 1.44)	1.02 (0.77, 1.37)
LDL-C (mmol/L)	2.99±0.85*	3.30±0.82*	2.92±0.78	3.22±0.78
UA (mmol/L)	0.37±0.08**	0.32±0.08**	0.35±0.08	0.28±0.06
Creatinine (mmol/L)	0.07±0.02	0.06±0.02**	0.07±0.02	0.05±0.01

*p<0.01, **p<0.001 vs non-MS for the same gender.
Values are shown as the mean±SD except for TG, which are shown as the median (1st, 3rd quartile). The survey was conducted from May 1, 2005 through to October 31, 2005.
MS, metabolic syndrome. Other abbreviations see Table 1.

of MS, with high BP being the most common component. It is also unclear how clinical profiles of metabolic parameters differ among each age group and gender in MS subjects.

With all this in mind, in the present study we clarified how the contribution of each component to the diagnosis of MS, according to the Japanese criteria, differed in each gender and age group by analyzing the cross-sectional data from the general population of Kanazawa city, Japan, who underwent a regular medical checkup.

Methods

A total of 21,870 people (7,329 men and 14,541 women) residing in Kanazawa city, Japan, who had an annual health examination from May 1, 2005 through to October 31, 2005, were involved in the study (Table 1). The participating subjects came from the general population and the examinations were conducted in hospitals located in Kanazawa city.

Study subjects were categorized into 5 groups according to age (years); namely, those in their 40s (In 40s: only age 40 and 45 were involved in the study), 50s, 60s, 70s, and 80 and older. When categorized further according to age group and gender, the number of men in those 5 groups were, respectively, 126; 815; 3,360; 2,442; and 586, respectively, and the number of women were 528; 2,844; 6,175; 3,884; and 1,110, respectively. MS was diagnosed based on the following Japanese criteria:[17–19] (1) waist circumference ≥85 cm for men or ≥90 cm for women; and (2) at least 2 of the following 3 components: TG ≥1.7 mmol/L and/or HDL-C <1.0 mmol/L, FPG ≥6.1 mmol/L and SBP ≥130 mmHg and/or DBP ≥85 mmHg.

Subjects fasted overnight before their blood samples were collected. Anthropometric parameters such as BP, body height and weight, and waist circumference were also measured on the same day. Total cholesterol and TG concentrations were measured enzymatically, as were HDL-C and low-density lipoprotein-cholesterol concentrations. Concentrations of FPG were determined using the glucose oxidase–oxygen electrode method. Hemoglobin A1c, excluding unstable fractions, was determined by high-pressure liquid chromatography. Informed consent was obtained from all subjects included in the study. Information regarding medication is lacking for all study subjects.

Statistical Analysis

Values are shown as the mean±SD unless otherwise noted. As serum TG did not distribute normally, data are expressed as the median (1st quartile, 3rd quartile). The frequency distribution of MS and central obesity was compared using the standard chi-squared test. Unpaired Student's t-test was conducted to compare parameters between MS and non-MS subjects. The TG values were logarithmically transformed before statistical analysis. Stat View 5.0 was used for statistical calculation. Probability values <0.05 were considered to be statistically significant.

Results

Overall Frequency of MS in the General Population and Values of Metabolic Parameters Between MS and Non-MS

The overall numbers of subjects with MS were 1,348 (18.4%) and 840 (5.8%) in men and women, respectively, in the studied general population (Table 2). Then, we compared metabolic parameters between MS and non-MS subjects for each gender. Almost all of the parameters, including serum uric acid (UA) levels, differed significantly between MS and non-MS for both genders with the exception that age and serum creatinine did not differ between MS and non-MS in men (Table 2).

Categorized further according to each age group and gender, the frequencies of MS were almost plateau across the various age groups in men, whereas frequencies of MS were higher in older women than in younger women (Fig 1). A similar tendency was observed for individuals with central obesity (waist circumference ≥85 cm for men and ≥90 cm for women) (Fig 2).

Contribution of Each Component to the Diagnosis of MS

We investigated how the metabolic components contributing to the diagnosis of MS differed among each age group for each gender. Across the genders and age groups, high BP was the most frequent contributor to the diagnosis of MS, followed by dyslipidemia, with high FPG being the least frequent contributor (Table 3). In contrast to this finding, isolated high DBP contributed very little to the diagnosis of MS. Similar findings were applied to isolated low HDL-C as a contributor to MS in both genders across the